

**Figure S1 (related to Figure 1).**

**A**, Kaplan-Meier survival curves for *PHF6* mutated patients according to mutation type in adverse and intermediate risk (ELN2017 classification) adult AML patients from the BEAT AML dataset. Statistical significance was calculated using the Log-rank (Mantel-Cox) test.

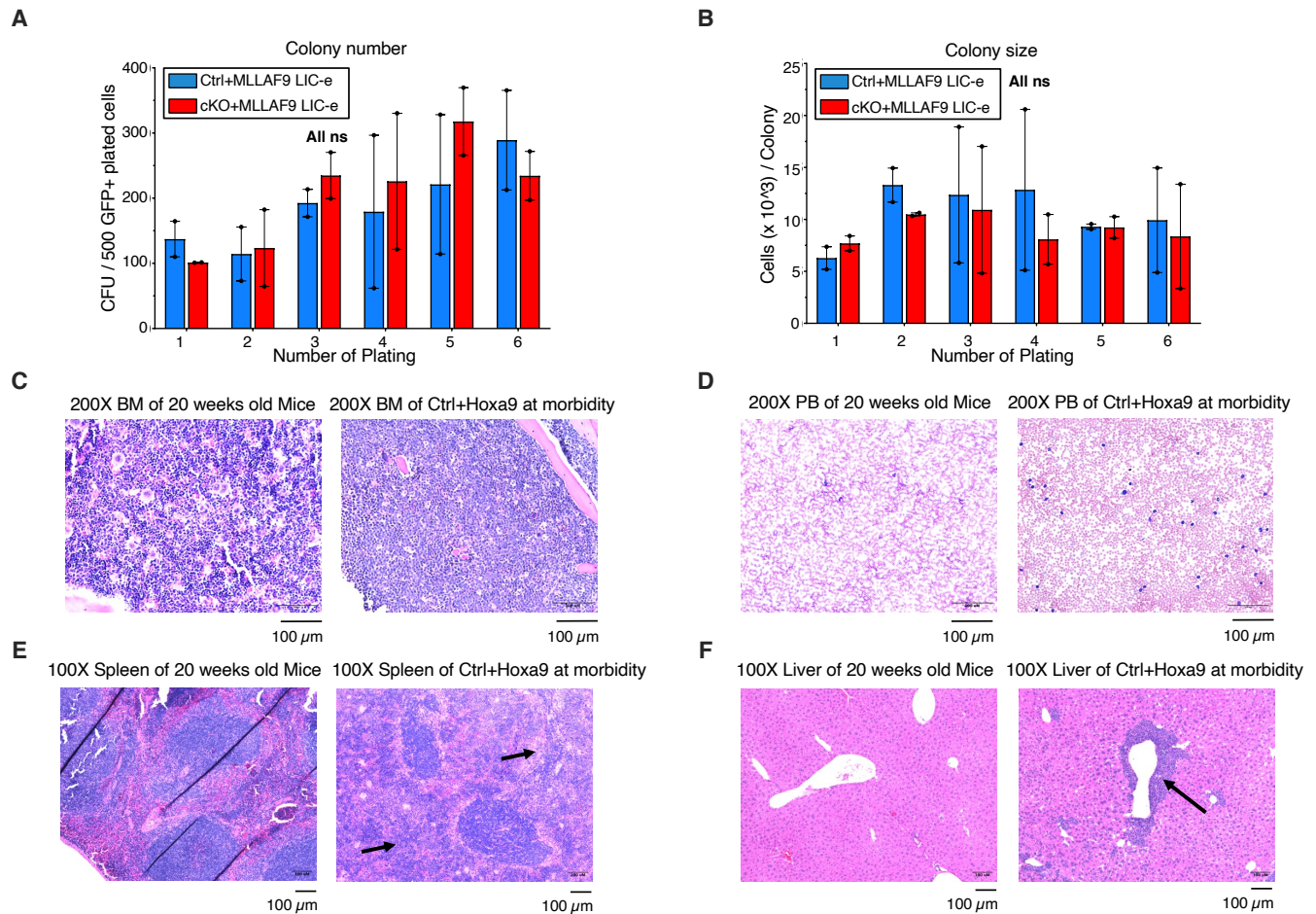
**B**, Lollipop mutation diagram representing different types of *PHF6* mutations in adverse, intermediate risk, and non-initiating (ELN2017 classification) adult AML patients from the BEAT AML dataset.

**C**, Tabular representation of different types of *PHF6* mutations in adverse and intermediate risk (ELN classification) adult AML patients from the BEAT AML dataset.

**D**, *Phf6* mRNA expression in bone marrow of *Vav-Cre<sup>Cre/+</sup> Phf6<sup>+/-</sup> (Ctrl)* and *Vav-Cre<sup>Cre/+</sup> Phf6<sup>fl/y</sup> (cKO)* mice. *Gapdh* is shown as a loading control. n = 3 biological replicates

**E-K**, Bar graphs depicting peripheral blood counts for WBCs, neutrophils, lymphocytes, monocytes, platelets, hemoglobin and RBCs for *Ctrl* and *cKO* mice at 8, 16, 24, and 40 weeks of age. n = 8 biological replicates

All bar graphs show mean  $\pm$  SEM. n.s. = non-significant by student t-test.



**Figure S2 (related to Figure 1).**

**A**, Bar graph showing number of colony forming units (CFUs) obtained from 6 rounds of serial methylcellulose replating of 500 cells/plate of Ctrl+MLL-AF9 and cKO+MLL-AF9 transformed mouse bone marrow. (n = 2 biological replicates)

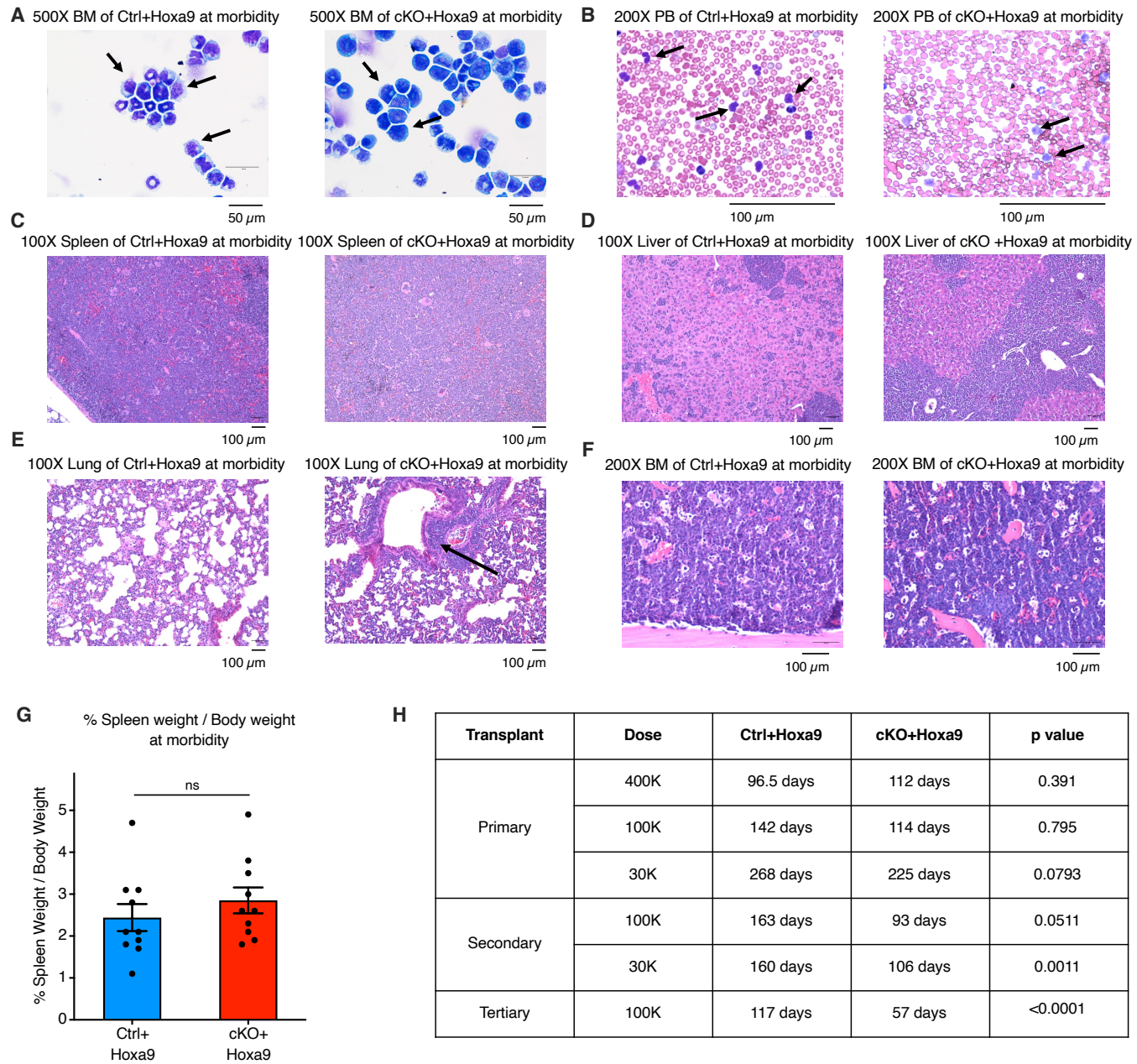
**B**, Bar graph showing average number of cells per colony (colony size) obtained after 6 rounds of serial methylcellulose replating of 500 cells/plate of Ctrl+MLL-AF9 and cKO+MLL-AF9 transformed mouse bone marrow. (n = 2 biological replicates).

**C**, H&E staining of bone marrow collected from a representative *Ctrl+Hoxa9* mouse at morbidity, with age-matched homeostatic (non-leukemic) WT mouse shown for reference. Scale bar is 100um at 200X.

**D**, Wright-Giemsa stain of blood smears from a representative *Ctrl+Hoxa9* mouse at morbidity, with age-matched homeostatic WT mouse shown for reference. Scale bar is 100um at 200X.

**E-F**, H&E staining of (**E**) spleen and (**F**) liver collected from a representative *Ctrl+Hoxa9* mouse at morbidity, with age-matched homeostatic (non-leukemic) WT mouse shown for reference. Scale bar is 100um at 100X. Arrows indicate leukemic infiltration.

All bar graphs show mean  $\pm$  SEM. n.s. = non-significant by student t-test.



**Figure S3 (related to Figure 1). Loss of Phf6 accelerates HoxA9-driven AML on serial transplantation**

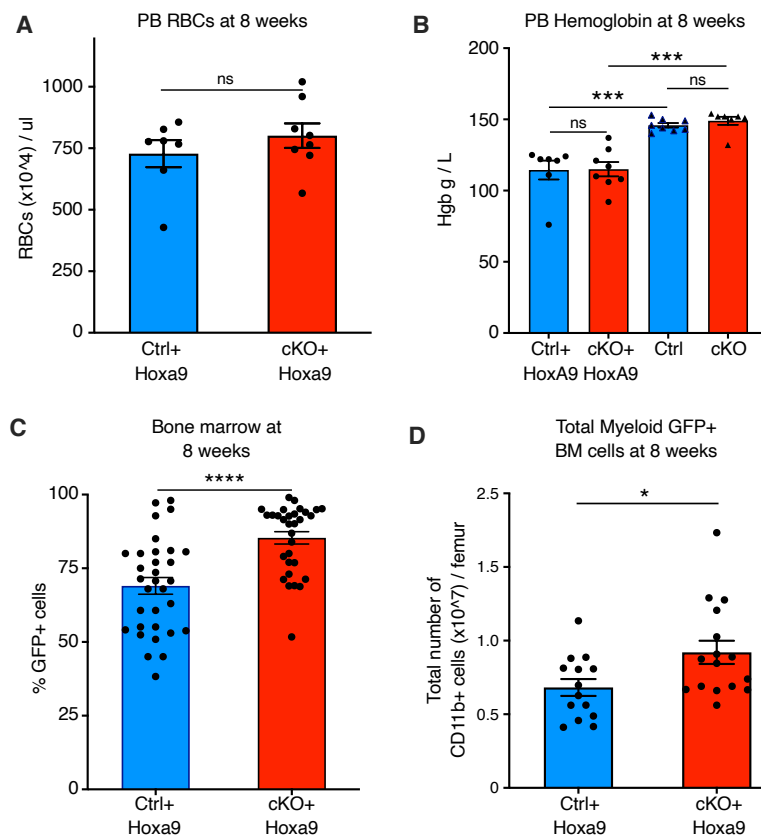
**A**, Representative image of Wright-Giemsa stain of bone marrow cytopins of *Ctrl+Hoxa9* and *cKO+Hoxa9* mice at morbidity. Scale bar is 50um at 500X. Arrows indicate blast cells.

**B**, Representative image of Wright-Giemsa stain of blood smears of *Ctrl+Hoxa9* mouse and *cKO+Hoxa9* mouse at morbidity. Scale bar is 100um at 200X. Arrows indicate blast cells.

**C-F**, Representative image of H&E staining of (**C**) spleen, (**D**) liver, (**E**) lung, and (**F**) bone marrow collected from *Ctrl+Hoxa9* mouse and *cKO+Hoxa9* mouse at morbidity. Scale bar is 100uM at 100X. Black arrows indicate leukemic infiltration.

**G**, Bar graph showing percent spleen / body weight at morbidity. Statistical significance was calculated using the Student t test. n.s. = non-significant by student t-test.

**H**, Table with median survival and statistical significance for primary, secondary and tertiary transplantation with different dose of *Ctrl+Hoxa9* and *cKO+Hoxa9* cells.

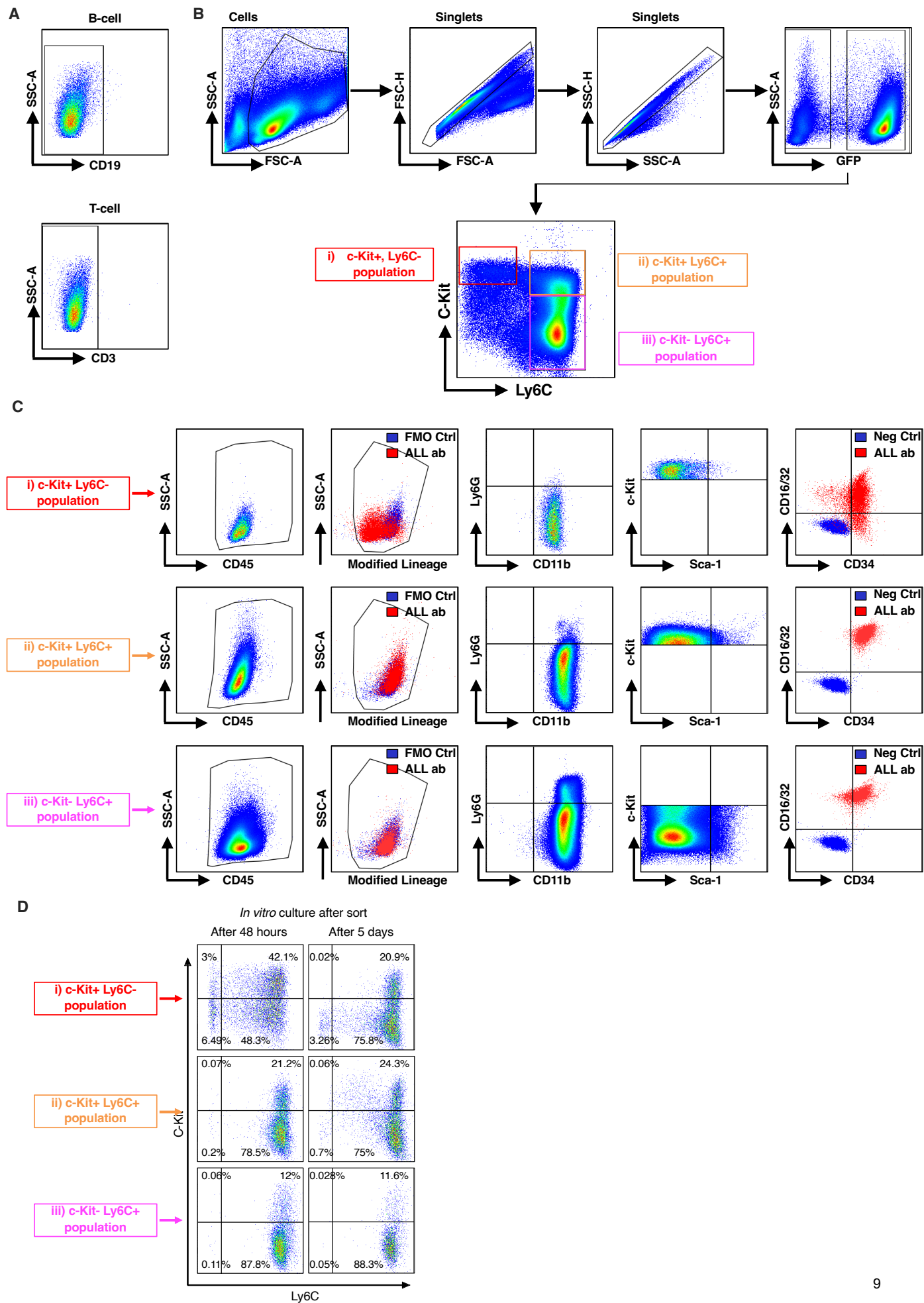


**Figure S4 (related to Figure 2). *Phf6* loss increases leukemic disease burden**

**A-B**, Bar graphs of peripheral blood counts for (**A**) RBCs and (**B**) hemoglobin level of *Ctrl+Hoxa9* and *cKO+Hoxa9* mice at 8 weeks, post primary transplantation. Hemoglobin levels of homeostatic (non-leukemic, non-transplanted) *Ctrl* and *cKO* mice are shown for reference. n = 7 biological replicates, n.s. = non-significant by student t-test.

**C-D**, Frequency of (**C**) GFP+ cells per femur and (**D**) total number of myeloid GFP+ cells per femur in the bone marrow of primary recipient after 8 weeks of transplantation of *Ctrl+Hoxa9* and *cKO+Hoxa9* cells.





**Figure S5 (related to Figure 3). Phenotypic characterization of *Hoxa9*-driven AML subpopulations**

**A**, Representative FACS plot of bone marrow leukemic cells (GFP+) from primary recipient mice at 8 weeks after transplantation. FACS plots demonstrate absence of B cell specific (CD19) and T cell specific (CD3) antigen expression.

**B-C**, FACS plots demonstrating the gating strategy used to compartmentalized GFP+ cells. We have designated the three sub-populations: red (**c-Kit+ Ly6C-**), orange (**c-Kit+ Ly6C+**) and pink (**c-Kit- Ly6C+**) as **LIC-e**, **Committed** and **Differentiated** leukemic cells respectively. Each population is further characterized for the expression of CD45 (pan hematopoietic marker), modified non-myeloid lineage cocktail (CD3, CD19, CD45R, Ter119, CD49b), CD11b (myeloid marker), Ly6G (neutrophil marker), c-kit, Sca-1, CD34, and CD16/32.

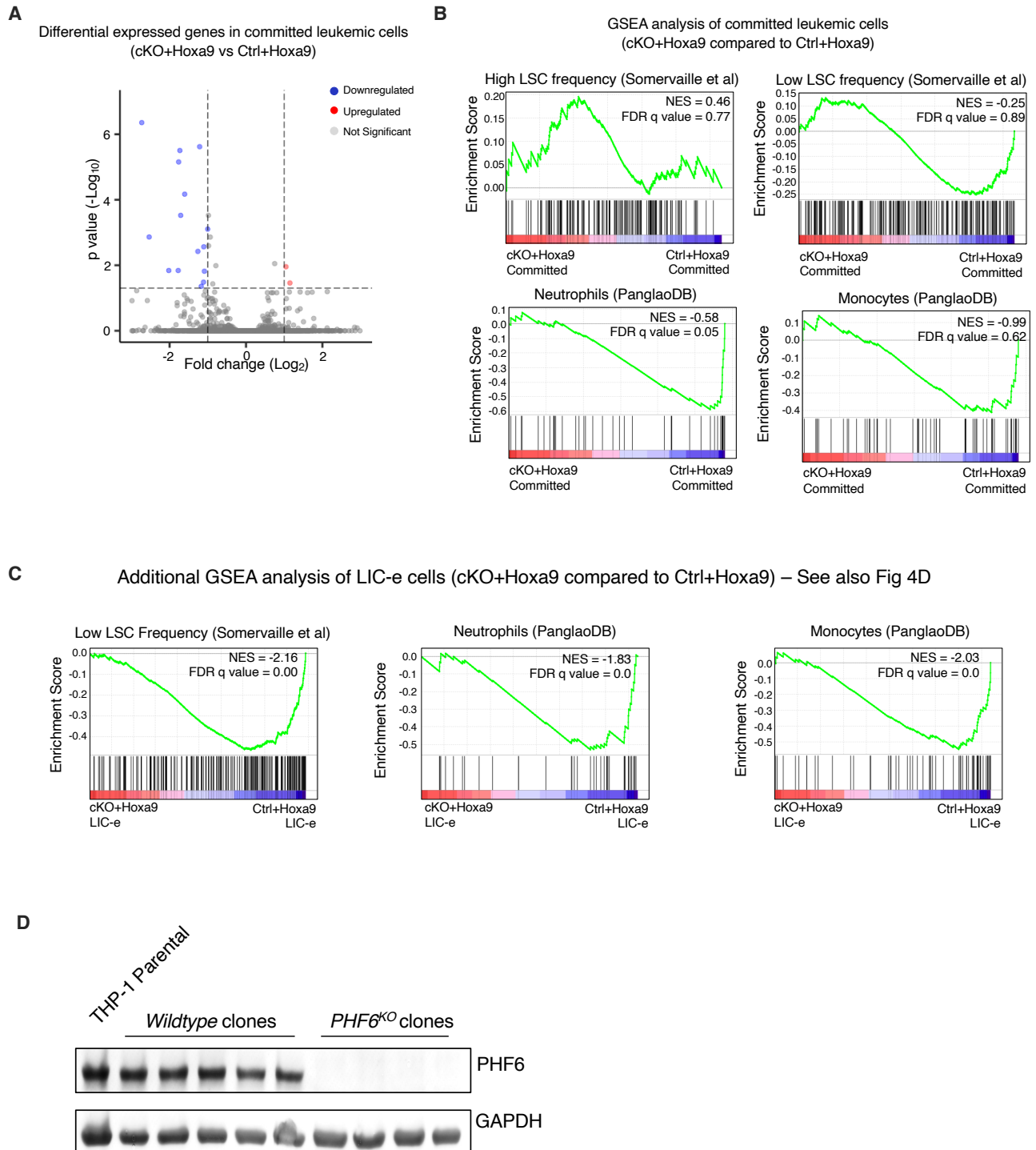
**LIC-e** cells were: mLin<sup>-</sup>Kit<sup>+</sup>Sca<sup>-</sup> CD11b<sup>dim</sup> Ly6G<sup>-</sup> CD34<sup>+</sup> CD16/32<sup>-/+</sup> Ly6C<sup>-</sup>

**Committed** cells were: mLin<sup>-</sup>Kit<sup>+</sup>Sca<sup>-</sup> CD11b<sup>+</sup> Ly6G<sup>-/+</sup> CD34<sup>+</sup> CD16/32<sup>+</sup> Ly6C<sup>+</sup>

**Differentiated** cells were: mLin<sup>-</sup>Kit<sup>-</sup>Sca<sup>-</sup> CD11b<sup>+</sup> Ly6G<sup>-/+</sup> CD34<sup>+</sup> CD16/32<sup>+</sup> Ly6C<sup>+</sup>

Note: The main flow plot in Fig S4B is also shown in main Fig 3A (shown here with detailed gating strategy).

**D**, Representative flow cytometry plots showing immunophenotypes of cells obtained after 48 hours and 5 days of *in vitro* culture of the three sorted subpopulations of *Ctrl+Hoxa9* transplanted marrow shown in Fig 3A.



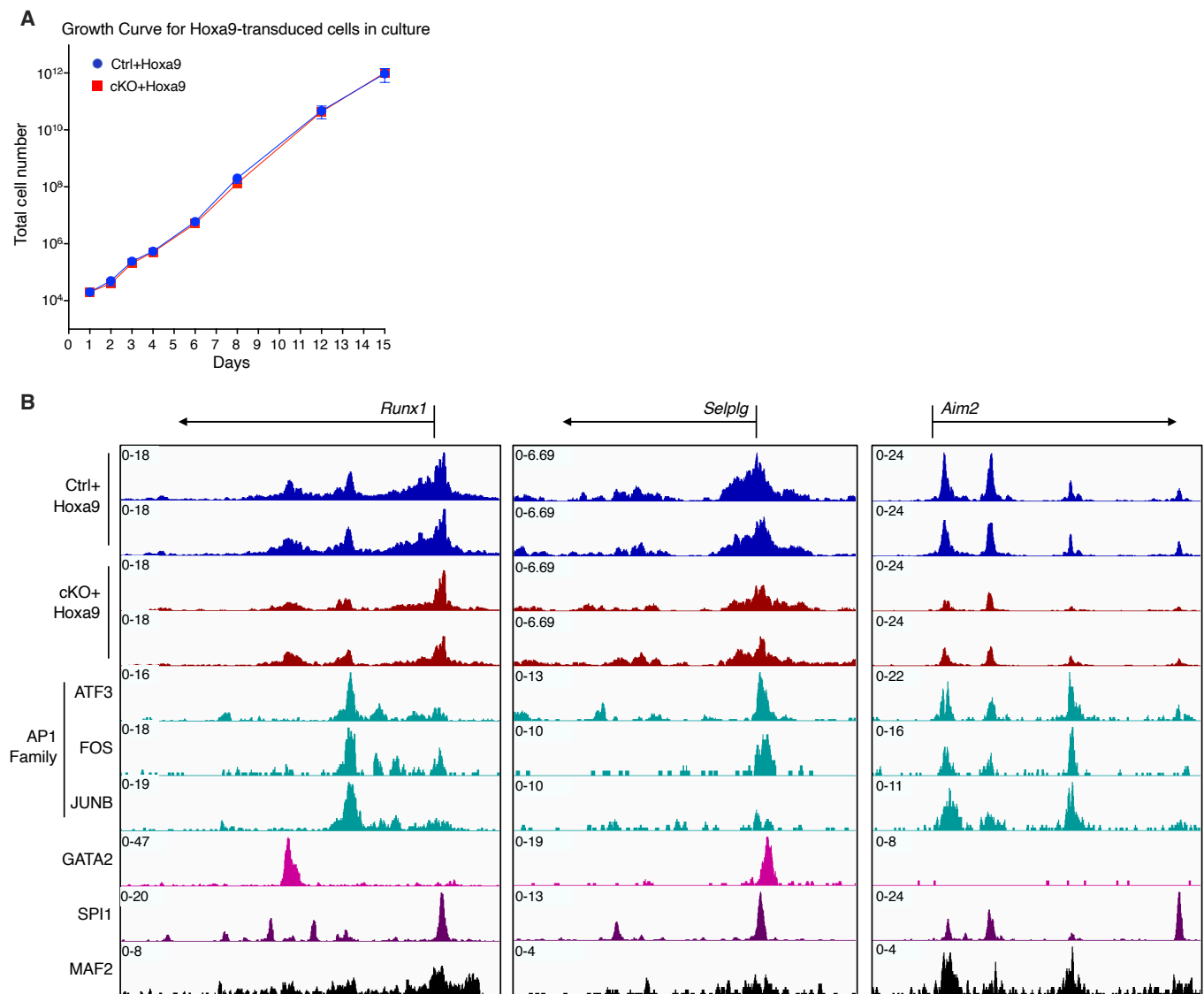
**Figure S6 (related to Figure 4). *Phf6* loss has minimal effects on the transcriptome of the committed (c-Kit<sup>+</sup>, Ly6C<sup>+</sup>) AML cell population**

**A**, Volcano plot showing differentially expressed genes between sorted committed cells (c-Kit<sup>+</sup>, Ly6C<sup>+</sup>) from *Ctrl+Hoxa9* and *cKO+Hoxa9* mice at 8 weeks after transplantation. Minimal gene expression changes were seen, with 28 downregulated and 3 upregulated genes in *cKO+Hoxa9* compared to *Ctrl+Hoxa9*.

**B**, Gene set enrichment analysis (GSEA) of the *cKO+Hoxa9* committed cell transcriptome compared to *Ctrl+Hoxa9* for gene sets related to high and low LSC frequency, mature neutrophils, and mature monocytes. Normalized Enrichment scores (NES) and FDR q values are shown.

**C**, Gene set enrichment analysis (GSEA) of the *cKO+Hoxa9* LIC-e transcriptome compared to *Ctrl+Hoxa9* for gene sets related to low LSC frequency, mature neutrophils, and mature monocytes. Normalized Enrichment scores (NES) and FDR q values are shown.

**D**, Immunoblot analysis verifying loss of PHF6 expression in human THP-1 *PHF6<sup>KO</sup>* clones compared to *wildtype* clones.



**Figure S7 (related to Figure 6). Tracks of transcription factor occupancy at differentially expressed genes**

**A**, Growth curve of *in vitro* cultured *Ctrl+Hoxa9* and *cKO+Hoxa9* cells.

**B**, IGV browser tracks of ATAC-Seq signal in LIC-e cells at promoters of genes downregulated or unchanged in *cKO+Hoxa9* along with ChIP-Seq signal of proteins whose motifs are enriched in regions of reduced accessibility in *cKO+Hoxa9* LIC-e cells.

*Ctrl+Hoxa9* and *cKO+Hoxa9* peaks are scaled to the same Y-axis. Transcription factor ChIP-Seq tracks are taken from publicly available datasets in myeloid or leukemic cell types (see Table S3).